1. FilmArray™ BioThreat-E Instructions for Use

For Use Under the Emergency Use Authorization (EUA) Only

Manufactured by BioFire Defense, LLC

Customer and Technical Support

Reach Us on the Web

http://www.BioFireDefense.com

Reach Us by Email support@BioFireDefense.com

Reach Us by Mail

79 West 4500 South, Suite 14
Salt Lake City, UT USA
84107

Reach Us by Phone

1-801-262-3592	US and Canada
1-801-262-3592	International

Reach Us by FAX

1-801-967-3688

BioFire Defense, LLC
79 West 4500 South, Suite 14
Salt Lake City, UT USA
84107

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Contents

Common Information

Intended Use Statement

The FilmArray Biothreat-E test is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic (IVD) test intended for the presumptive detection of Ebola Zaire virus (detected in the West Africa outbreak in 2014) in whole blood specimens or undiluted urine specimens. The FilmArray Biothreat-E test is performed on the FilmArray Instrument to detect RNA from the Ebola Zaire virus in specimens from individuals with signs and symptoms of Ebola virus infection in conjunction with epidemiological risk factors.

Testing with the FilmArray Biothreat-E test should not be performed unless the individual has signs and symptoms of infection with Ebola Zaire that meet clinical and epidemiologic criteria for testing suspect specimens.

Test results are for the presumptive identification of Ebola Zaire virus. The definitive identification of Ebola Zaire virus requires additional testing and confirmation procedures in consultation with public health or other authorities for whom reporting is required. The diagnosis of Ebola virus infection must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the identification of the Ebola Zaire virus. The level of Ebola Zaire virus that would be present in blood or urine from individuals with early infection is unknown. Due to the difficulty in obtaining clinical specimens, this test was evaluated with limited numbers of contrived specimens spiked with inactivated Ebola Zaire virus and limited numbers of blood and urine specimens from individuals infected with the Ebola Zaire virus. Negative results do not preclude Ebola Zaire virus infection and should not be used as the sole basis for patient management decisions.

The FilmArray BioThreat-E test is for use only under Emergency Use Authorization (EUA) by laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate complexity tests and by laboratories certified under CLIA to perform high complexity tests and by clinical laboratory personnel that have been appropriately trained.

Notification of Public Health: Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection

OR no detection EVD test result on the need for additional testing and appropriate transportation of specimens.

Introduction

The FilmArray BioThreat-E test has been authorized for use under an EUA on the FilmArray instrument platform to provide Ebola Zaire virus testing capability to clinical sites that currently perform moderate complexity tests and by laboratories certified under CLIA to perform high complexity tests with the FilmArray System.

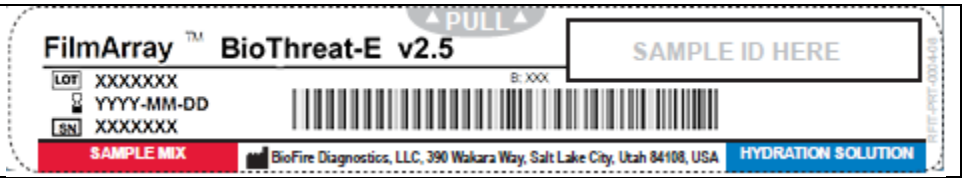
All users, analysts, and persons reporting diagnostic results from the FilmArray BioThreat-E test should be trained in proper use of the instrument and software.

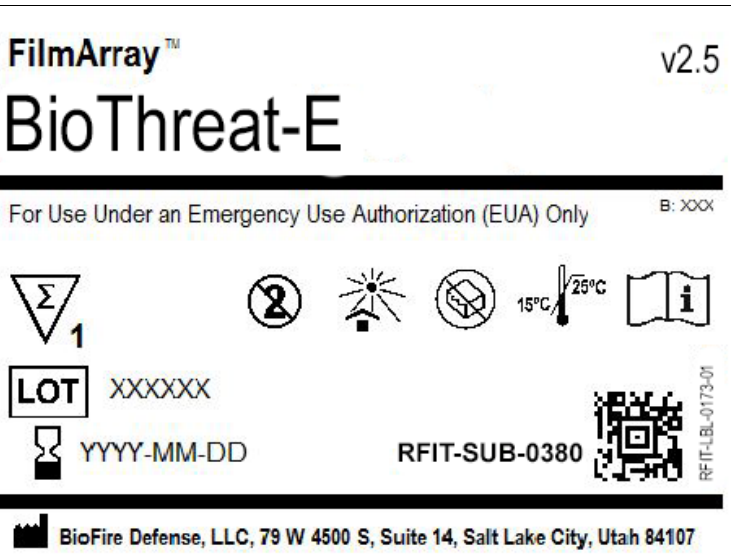
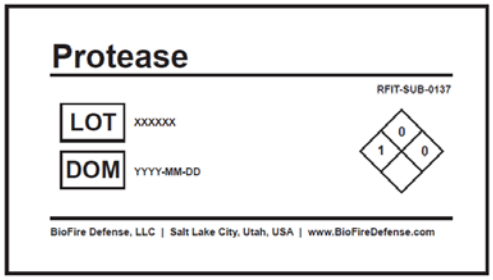
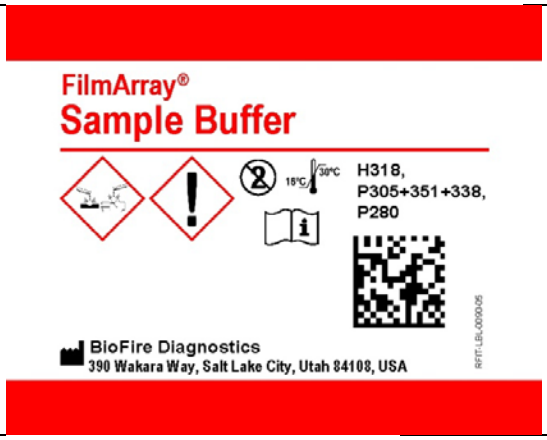
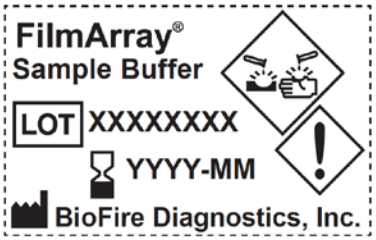
Storage of Contents

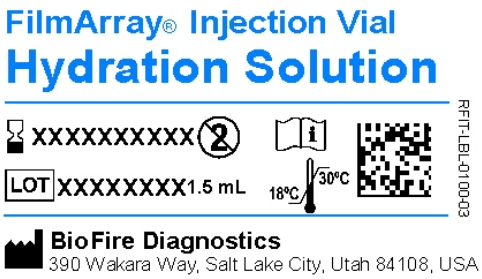
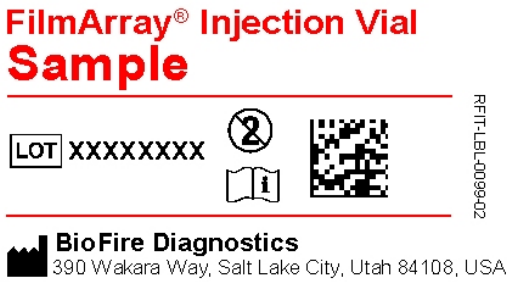
1. Store the test kit, including reagent pouches and buffers, at room temperature (15-25 ºC). **DO NOT REFRIGERATE.**
2. Avoid storage of any materials near heating or cooling vents or in direct sunlight.
3. Always check the expiration date and do not use reagents beyond the expiration date printed on the pouch or kit.
4. Do not remove pouches from their packaging until a sample is ready to be tested. Once the pouch packaging has been opened, the pouch should be loaded as soon as possible (within approximately 30 minutes).
5. Once a pouch has been loaded, the test run should be started as soon as possible (within 60 minutes).

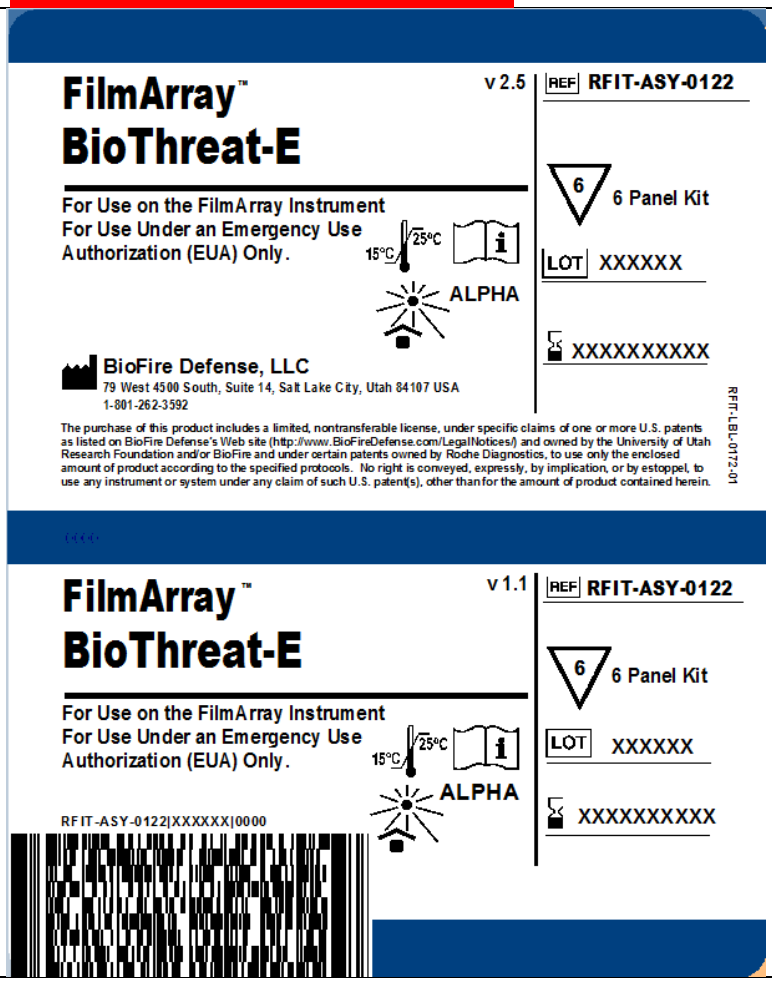
Kit Labels

Examples of labels found with the FilmArray BioThreat-E Kit are:

Fitment Label	

Mylar Pouch Label	
Protease Vial Label	**Protease** **LOT: XXXXXX** **DOM: YYYY-MM-DD** **BioFire Defense, LLC**
Mylar pouch label for the protease vial	
FilmArray Sample Buffer Ampoule Bag Label – 7 Pack	
Sample Buffer Ampoule Label	

Hydration Solution Injection Vial Bag Label – 7 Pack	
Hydration Solution Injection Vial Label	
Sample Buffer Injection Vial Label	
Sample Buffer Injection Vial Bag Label – 7 Pack	

Transfer Pipette Bag Label – 7 Pack	
Outer Kit Box Label	

Materials Provided

Each kit contains sufficient reagents to test 6 samples:

- Individually packaged FilmArray BioThreat-E pouches
- Single-use (1.0 mL) Sample Buffer ampoules

- Single-use freeze-dried protease vials
- Single-use pre-filled (1.5 mL) Hydration Injection Vials (blue)
- Single-use Sample Injection Vials (red)
- Individually packaged Transfer Pipettes

Materials Required But Not Provided

- FilmArray System with laptop computer
- FilmArray Pouch Loading Station compatible with the use of the FilmArray Injection Vials (included with FilmArray System)
- Bleach
- De-ionized water

Acceptable Specimens

- Whole Blood
- Urine
    - **NOTE:** Urine should not be the sole specimen tested from a patient. If a urine specimen from a patient is tested, it must be tested in conjunction with a whole blood specimen from the patient.

Principle of the Procedure

The following is an overview of the operations and processes that occur during a FilmArray run:

1. **Nucleic Acid Purification** - Nucleic acid purification occurs in the first three blisters of the pouch. The sample is lysed by a combination of chemical and mechanical (bead beating) mechanisms and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require about ten minutes, and the bead-beater apparatus can be heard as a high-pitched whine during the first few minutes of operation.

2. **Reverse Transcription and 1st Stage Multiplex PCR** - Since the Ebola virus has an RNA genome, a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the RT step and subsequent thermocycling for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

3. **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye (LCGreen™ Plus, BioFire Defense, LLC.). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

4. **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or T_m) is consistent and predictable and the FilmArray Software automatically evaluates the data from replicate wells for each assay to report results.

The FilmArray Software controls the operation of the instrument, collects and analyzes data, and automatically generates a test report at the end of the run. The entire process takes about an hour. Additional detail can be found in the FilmArray Operator's Manual.

Testing Procedure

> **NOTE:** Testing should be performed under the appropriate biosafety conditions and in accordance with CDC guidelines. This information can be found in: *Infection Control for Viral Hemorrhagic Fevers in the African Health Care Setting*, developed by the U.S. Centers for Disease and Prevention (CDC) in conjunction with the World Health Organization (WHO) and found at http://www.cdc.gov/vhf/abroad/healthcare-workers.html and in: Information for Healthcare Workers in the United States: http://www.cdc.gov/vhf/ebola/hcp/.

Refer to the FilmArray BioThreat-E Quick Guide, for abbreviated pictorial representations of these instructions.

Gloves and other Personal Protective Equipment (PPE) should be used when handling pouches and samples. Only one FilmArray pouch should be prepared at a time. Once sample is added to the pouch, it should be promptly transferred to the instrument to start the run. After the run is complete, the pouch should be discarded in a biohazard container.

Prepare Pouch

1. Thoroughly clean the work area and the FilmArray Pouch Loading Station with freshly prepared 10% bleach (or suitable disinfectant) followed by a water rinse. Note: Ensure

that the Pouch Loading Station is compatible with the use of the FilmArray Injection Vials.

2. Obtain the following required materials and place in the clean hood:
    - FilmArray BioThreat-E Assay pouch
    - Sample buffer ampoule
    - Protease vial
    - Hydration Injection Vial (blue cap)
    - Sample Injection Vial (red cap)
    - Transfer pipette
3. Place a blue capped Hydration Injection Vial in the blue well of the Pouch Loading Station.
4. Place a red capped Sample Injection Vial in the red well of the Pouch Loading Station.
5. Obtain patient sample and place into hood.
6. Remove the FilmArray pouch from its vacuum-sealed package by tearing or cutting the notched outer packaging and opening the protective aluminum canister.

> **NOTE: If the vacuum seal of the pouch is not intact, the pouch may still be used. Attempt to hydrate the pouch using the steps in the Hydrate Pouch section. If hydration is successful, continue with the run. If hydration fails, discard the pouch and use a new pouch to test the sample.**

7. Slide the pouch into the Pouch Loading Station so that the red and blue labels on the pouch align with the red and blue arrows on the base of the Pouch Loading Station.

Hydrate Pouch

1. Twist the Hydration Injection Vial (blue cap), leaving cap in Pouch Loading Station, and insert the tip of the cannula into the hydration port of the pouch located directly below the blue arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
2. Verify that the pouch has been hydrated. Flip the barcode label down and check to see that fluid has entered the reagent wells (located at the base of the rigid plastic part of the pouch). Small air bubbles may be seen. If the pouch fails to hydrate (dry reagents appear as white pellets), verify that the seal of the port was broken by ensuring the vial cannula

was fully inserted into the hydration port. If the pouch fails to hydrate, retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.

3. Discard the Hydration Injection Vial in a suitable puncture proof container.

Prepare Sample Mix- WHOLE BLOOD

1. Hold the Sample Buffer ampoule so that the tip is facing up.

> **NOTE: Use care to avoid touching the tip during handling, as this may introduce contamination.**

2. Gently pinch the textured plastic tab on side of ampoule until the seal snaps.
3. Re-position thumb and forefinger to grip between the textured plastic tab and the bottom of the ampoule, then invert over the un-capped Protease vial and dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.
4. Re-cap Protease Vial and invert 3 times to mix.
5. Un-cap Protease Vial and pour buffer/protease mixture into red Sample Injection Vial.
6. Draw specimen up to the second line of the transfer pipette (~200 µL) and add to Sample Injection Vial.
7. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least 3 times.
8. Return the Sample Injection Vial to the Pouch Loading Station.

Prepare Sample Mix- URINE

1. Hold the Sample Buffer ampoule so that the tip is facing up.

> **NOTE: Use care to avoid touching the tip during handling, as this may introduce contamination.**

2. Gently pinch the textured plastic tab on side of ampoule until the seal snaps.
3. Re-position thumb and forefinger to grip between the textured plastic tab and the bottom of the ampoule, then invert over the Sample Injection Vial dispense Sample Buffer using a slow, forceful squeeze, followed by a second squeeze. Avoid generating excessive bubbles.
4. Draw specimen up to the second line of the transfer pipette (~200 µL) and add to the Sample Injection Vial.

5. Tightly close the lid of the Sample Injection Vial and mix by gently inverting at least 3 times.
6. Return the Sample Injection Vial to the Pouch Loading Station.

Load Sample Mix

1. Slowly unscrew Sample Injection Vial from the cap and pause for 3-5 seconds.

   NOTE: It is important to pause after unscrewing the Sample Injection Vial to avoid sample leakage and contamination of the work area.

2. Remove Sample Injection Vial leaving cap in Pouch Loading Station and insert the cannula tip into the port in the pouch fitment located directly below the red arrow of the Pouch Loading Station. Push down forcefully in a firm and quick motion until you hear a faint “pop” and feel an ease in resistance. The correct volume of liquid will be pulled into the pouch by vacuum.
3. Verify that the sample has been loaded. Flip the barcode label down and check to see that fluid has entered the reagent well next to the sample loading port. If the pouch fails to pull sample from the Sample Injection Vial, the pouch should be discarded. Retrieve a new pouch and repeat from Step 2 of the Prepare Pouch section.
4. Discard the Sample Injection Vial in a suitable biohazard and puncture proof container.
5. Record the Sample ID in the provided area on the pouch label (or affix a barcoded Sample ID) and remove the pouch from the Pouch Loading Station.

Run Pouch

The FilmArray Instrument Control Software includes a step-by-step on-screen tutor that shows each step of the test.

1. Ensure that the computer and FilmArray Instrument have been turned on. Launch the FilmArray Instrument Control Software by double clicking on the desktop icon.
2. Open the instrument lid (if not already open).
3. Insert the FilmArray pouch into the instrument.

   Position the pouch so that the array is on the right with the film directed downward into FilmArray Instrument. The red and blue labels on the FilmArray pouch should align with the red and blue arrows on the FilmArray Instrument. The pouch will click into place. If

inserted correctly, the barcode is visible and the label is readable on the top of the pouch. The instrument and software must detect that the pouch has been inserted correctly before continuing to the next step.

NOTE: If the pouch does not slide into the instrument easily, gently push the lid of the instrument back to be sure that it is completely open.

4. Scan the barcode on the FilmArray pouch using the barcode scanner.

   Pouch identification (Lot Number and Serial Number) and Pouch Type are preprogrammed in the barcode located on the FilmArray pouch and will be automatically entered when the barcode is scanned. If it is not possible to scan the barcode, the pouch Lot Number, Serial Number, and Pouch Type can be manually entered from the information provided on the pouch label. To reduce data entry errors, it is strongly recommended that the pouch information be entered by scanning the barcode.

   NOTE: The barcode cannot be scanned prior to placing the pouch in the instrument. A "Cannot scan now" message will be displayed.

5. Enter the Sample ID.

   The Sample ID can be entered manually or scanned in by using the barcode scanner when a barcoded Sample ID is used.

6. Select the Blood protocol (for whole blood samples) or Urine protocol (for urine samples) from the Protocol drop down list.
7. Enter a user name and password in the Name and Password fields.
8. Close the FilmArray Instrument lid.
9. Click Start Run.

   Once the run has started, the screen displays a list of the steps being performed by the instrument and the number of minutes remaining in the run.

   NOTE: The bead-beater apparatus can be heard as a high-pitched noise (whine) during the first few minutes of operation.

10. When the run is finished, results are automatically displayed in the report section of the screen. The report is automatically saved into the database.
11. Select **Print** to print the report, or **Save** to save the report as a PDF file.
12. Follow the on-screen instructions to open the instrument and remove the pouch.

Immediately discard the pouch in a biohazard container.

Quality Control

RNA Process Control

The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. The yeast is present in the pouch in a freeze-dried form and becomes rehydrated when sample is loaded. The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray BT pouch were successful.

The process control assay must be positive for the test run to pass. If the control fails, the Control field of the test report (upper right hand corner) will display "Failed" and all results will be listed as "Invalid". If the control fails, the sample should be retested using a new pouch.

Note that for the BioThreat-E Test a DNA Process Control and PCR2 Control are visible in the data analysis screen, but these controls are not used to evaluate data.

External Controls

Information on how to obtain optional external control material is posted on the BioFire Defense, website at http://biofiredefense.com/support/filmarray-support/

Assay Interpretation

When 2nd stage PCR is complete, the FilmArray Instrument performs a high resolution DNA melting analysis on the PCR products and measures the fluorescence signal generated in each well (for more information see FilmArray Operator's Manual). The FilmArray Software then performs several analyses and assigns a final assay result. The steps in the analysis are described below.

Analysis of melt curves. The FilmArray Software evaluates the DNA melt curve for each well of the 2nd stage PCR array to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature (Tm) of the curve. The Tm value is then compared against the expected Tm range for the assay. If the software determines that the melt curve is positive and the Tm falls inside the assay-specific Tm range, the melt curve is called positive. If the software determines that the melt curve is negative or is not in the appropriate Tm range, the melt curve is called negative.

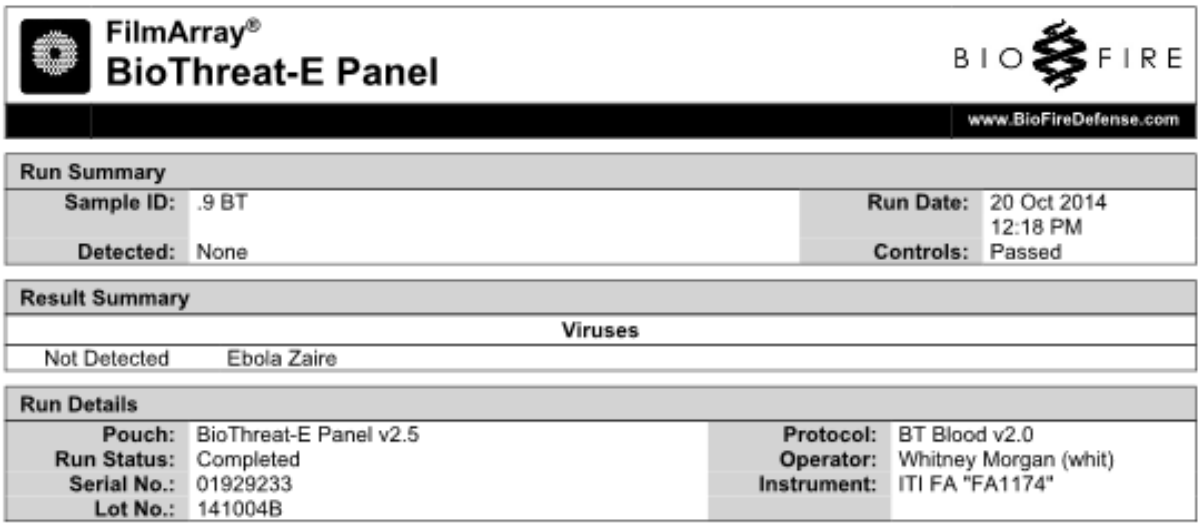
Analysis of replicates. Once melt curves have been identified, the software evaluates the three replicates for each assay to determine the assay result. For an assay to be called positive, at least two of the three associated melt curves must be called positive, and the Tm for at least two of the three positive melt curves must be similar (within 1°C). Assays that do not meet these criteria are called negative.

FilmArray Test Report

The FilmArray BioThreat-E assay test report is automatically displayed upon completion of a run and contains three sections, the Run Summary, the Result Summary, and the Run Details. The test report can be saved as a PDF or printed. Examples of negative (Figure 1) and positive (

Figure 2) run reports are shown below.

Figure 1. Example test report of a negative FilmArray run.



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Run Summary

Sample ID:	.9 BT	**Run Date:**	20 Oct 2014 12:18 PM
Detected:	None	**Controls:**	Passed

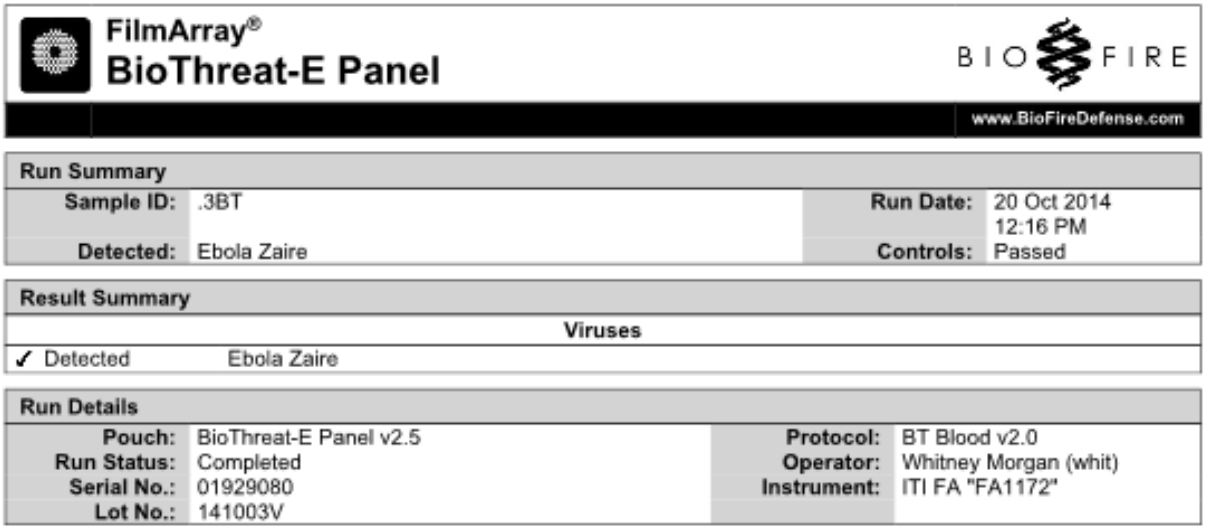
Result Summary

	Viruses
Not Detected	Ebola Zaire

Run Details

Pouch:	BioThreat-E Panel v2.5	**Protocol:**	BT Blood v2.0
Run Status:	Completed	**Operator:**	Whitney Morgan (whit)
Serial No.:	01929233	**Instrument:**	ITI FA "FA1174"
Lot No.:	141004B		

Figure 2. Example test report of a positive FilmArray run.



FilmArray®
BioThreat-E Panel

BIOFIRE

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Run Summary

Sample ID:	.3BT	**Run Date:**	20 Oct 2014 12:16 PM
Detected:	Ebola Zaire	**Controls:**	Passed

Result Summary

	Viruses
✓ Detected	Ebola Zaire

Run Details

Pouch:	BioThreat-E Panel v2.5	**Protocol:**	BT Blood v2.0
Run Status:	Completed	**Operator:**	Whitney Morgan (whit)
Serial No.:	01929080	**Instrument:**	ITI FA "FA1172"
Lot No.:	141003V		

The **Run Summary** section of the test report provides the Sample ID, time and date of the run, control results and an overall summary of the test results. An “Ebola Zaire” result will be listed in the “Detected” field of the summary. If the test is negative then “None” will be displayed in the “Detected” field. The control is listed as “Passed”, “Failed” or “Invalid”. See the Controls Field section below for detailed information about the interpretation of controls and appropriate follow-up in the case of control failures.

The **Result Summary** section of the test report lists the result for Ebola Zaire. Possible results are “Detected”, “Not Detected”, or “Invalid”. See the Results Summary - Interpretations section below for detailed information about interpretation of test results and appropriate follow-up for Invalid results.

The **Run Details** section provides additional information about the run including: pouch information (type, lot number, and serial number), Run Status (Completed, Incomplete, Aborted, Instrument Error, Instrument Communication Error, or Software Error), the protocol that was used to perform the test, the identity of the operator that performed the test, and the instrument used to perform the test.

Once a run has completed, it is possible to edit the Sample ID. If this information has been changed, an additional section called **Change History** will be added to the test report. This Change History section lists the field that was changed, the original entry, the revised entry, the operator that made the change, and the date that the change was made. Sample ID is the only field of the report that can be changed.

Control Field

The Control field on the test report will display “Passed”, “Failed”, or “Invalid”. The Control field will display “Passed” only if the run completed successfully (no instrument or software errors) and the pouch control assay (RNA Process Control) was successful. The Control field will display “Failed” if the run was completed successfully (no instrument or software errors) but the pouch control assay failed. The Controls field will display “Invalid” if the run did not complete (typically indicates a software or hardware error). If the control result is “Failed” or “Invalid”, then the result for Ebola Zaire is displayed as “Invalid” and the sample will need to be retested with a new pouch.

Table 1 provides a summary and explanation of the possible control results and follow-up actions.

Table 1. Interpretation of Controls Field on the FilmArray BioThreat-E Test Report

Control Result	Explanation	Action Required	Outcome
Passed	The run was successfully completed AND The pouch control was successful.	None	Report the results provided on the test report.
Failed	The run was successfully completed BUT The pouch control (RNA Process Control) failed.	Repeat the test using a new pouch.	Accept the results of the repeat testing. If the error persists, contact Technical Support for further instruction.
Invalid	The control is invalid because the run did not complete. (Typically this indicates a software or hardware error).	Note any error codes displayed during the run and the Run Status field in the Run Details section of the report. Refer to the FilmArray Operator’s Manual or contact Technical Support for further instruction. Once the error is resolved, repeat the test or repeat the test using another instrument.	Accept the valid results of the repeat testing. If the error persists, contact Technical Support for further instruction.

Results Summary-- Interpretations

The Results Summary – Interpretations section lists the Ebola Zaire result. Possible results include “Detected”, Not “Detected”, and “Invalid”. Table 2 provides an explanation for each interpretation and any follow-up actions necessary to obtain a final result.

Table 2. Reporting of Results and Required Actions

Result	Explanation	Action
Detected	The run was successfully completed AND The pouch control was successful (Passed) AND The assay(s) associated with the interpretation were positive based on the following requirements for at least 2 of the 3 assay replicates: -a positive melt curve, and -the Tm for the melt data were within the assay specific limits, and -the Tm for the melt data were within 1°C of each other.	None. Report results.
Not Detected	The run was successfully completed AND The pouch control was successful (Passed) AND The assay(s) associated with the interpretation were negative (did not meet the requirements for a positive assay described in Detected).	None. Report results.
Invalid	The run did not complete successfully (Aborted, Incomplete, Instrument Communication Error, Instrument Error, or Software Error) OR The pouch control was not successful (Failed)	See Table 1, *Interpretation of Controls Field on FilmArray Report*, for instruction.

Assay Limitations

- Negative test results do not preclude infection with Ebola virus and should not be the sole basis of patient treatment/management decisions.
- This test should not be used to test specimens from asymptomatic individuals
- This product can only be used with the FilmArray Instrument
- This test is a qualitative test and does not provide a quantitative value for the virus in the sample.
- This test has been evaluated for use with human whole blood and urine material only.
- All results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms.

- Interpretation of results from the FilmArray BioThreat-E test must account for the possibility of false-positive and false-negative results.
- False positive results may occur from cross-contamination by target organism, their nucleic acids, or from PCR amplicon.
- Failure to follow assay procedures may lead to false-negative results.
- Inhibitors present in the samples may lead to false-negative results.
- Specimens from patients who have received therapeutics or vaccines based on nucleic acid sequences derived from Ebola Zaire virus may exhibit false positive or other confounding test results.

Warnings and Precautions

- For *in vitro* diagnostic use under Emergency Use Authorization only.
- Local, state, and national public health agencies (for example, county and state health departments or the U.S. Centers for Disease Control and Prevention (CDC)) should be notified of any patient suspected to have Ebola Virus Disease (EVD). Confirmatory testing at the state/local public health laboratory or at CDC is necessary for positive detection results and may be necessary for negative detection results. Laboratories should consult with local, state or national public health officials on any positive detection OR no detection (negative) EVD test result on the need for additional testing and appropriate transportation of specimens.
- All results should be interpreted by a trained professional in conjunction with review of the patient's clinical signs and symptoms and history.
- Use of this assay should only be for trained personnel.
- Treat all specimens as potentially infectious.
- Follow necessary precautions when handling samples and reagents.
- Performance of the FilmArray BioThreat-E test has only been evaluated for the specimen types described in the Intended Use.
- Proper sample collection, storage, and transport are essential for valid test results.
- Do not use reagents from other manufacturers with this assay.
- Use appropriate laboratory and personal protective equipment when using this kit.

Performance Characteristics

NOTE:

The FilmArray BioThreat-E test (v2.5) has been incrementally optimized (by adding primer sequence degeneracy) to increase analyte detection efficiency and improve detection of the Ebola Zaire virus currently in circulation in the 2014 West African outbreak. A subset of the performance data presented below was collected using previous versions of the test (v2.2-2.4).

Analytical Sensitivity/Limit of Detection (LoD)

An estimated Limit of Detection of 6.00 E+05 plaque-forming units (PFU)/mL was determined using inactivated Ebola Zaire virus in whole blood. Testing was performed on 200 µL aliquots in quadruplicate. Confirmation of the Limit of Detection was done by confirming the 95% detection rate with 20 replicates.

Analytical Reactivity

Due to limitations in acquiring inactivated stocks of Ebola Zaire virus, reactivity of FilmArray BioThreat-E test was not evaluated with any non-Mayinga strains of Ebola Zaire.

However, *in silico* analysis of the primer sequences and the available corresponding target gene sequences (n=30 for non-outbreak strain gene targets, n=99 for current 2014 outbreak strain target gene sequences) was performed. For all 30 non-outbreak strain target sequences, the primer sequences and annealing regions were found to be identical. For the 99 2014 outbreak strain target sequences, all 99 were identical to the primer sequences.

In addition, the ability of FilmArray BioThreat-E test to detect the Ebola Zaire strain currently circulating in West Africa was evaluated using a synthetic template (gBlock, IDT, Coralville, IA, USA) derived from the ZEBOV.Guinea.2014 sequence (Accession: KJ660346), which was used to generate Ebola Zaire L-gene RNA. *In vitro* transcribed RNA was quantified and spiked into TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) at concentrations varying from 1.00E+00 to 1.00E+05 genomic equivalents (GE) per reaction in duplicate. Detection was observed for all samples tested.

Analytical Specificity (Cross-reactivity)

FilmArray BioThreat-E test was evaluated for potential cross-reactivity by testing an extensive list of bacterial and viral strains. Two similar evaluations (Table 3 and Table 4) were performed on earlier (pre-v2.5) versions of the FilmArray BioThreat-E test where the primers contained fewer sequence degeneracies than the current version (v2.5).

Table 3 Cross-Reactivity Organism Set 1 (FilmArray BioThreat-E v2.2)

Organism/Virus	ID #	Sample & Matrix (SB[1] or WB[2])	Concentration	BioThreat-E Result
Acinetobacter baumanni	ACIN001	Nucleic Acid/ SB	Unknown	Not Detected
Bacillus anthracis	BACI056	Live organism/ WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI002	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI008	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI124	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI126	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI153	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI155	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI207	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI224	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI225	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI259	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI261	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus anthracis	BACI293	Live organism / WB	5.00+02 CFU/mL	Not Detected
Bacillus cereus	BACI015	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI016	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI227	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI228	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI290	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI232	Nucleic Acid / SB	Unknown	Not Detected
Bacillus cereus	BACI234	Nucleic Acid / SB	Unknown	Not Detected
Bacillus megaterium	BACI026	Nucleic Acid / SB	Unknown	Not Detected
Bacillus mycoides	BACI028	Nucleic Acid / SB	Unknown	Not Detected
Bacillus mycoides	BACI088	Nucleic Acid / SB	Unknown	Not Detected
Bacillus subtilis	BACI051	Nucleic Acid / SB	Unknown	Not Detected
Bacillus subtilis var niger	BACI034	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringieinsis	BACI036	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringieinsis	BACI052	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringieinsis	BACI172	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringiensis	BACI265	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringiensis	BACI204	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringiensis	BACI229	Nucleic Acid / SB	Unknown	Not Detected
Bacillus thuringiensis	BACI230	Nucleic Acid / SB	Unknown	Not Detected
Brucella abortus	BRUC012	Live organism / WB	5.00+01 CFU/mL	Not Detected
Brucella abortus	BRUC088	Live organism / WB	5.00+02 CFU/mL	Not Detected
Brucella abortus	BRUC087	Live organism / WB	5.00+02 CFU/mL	Not Detected
Brucella abortus	BRUC096	Live organism / WB	5.00+02 CFU/mL	Not Detected
Brucella abortus	BRUC095	Live organism / WB	5.00+02 CFU/mL	Not Detected
Brucella melitensis	BRUC013	Live organism / WB	5.00+01 CFU/mL	Not Detected
Brucella ovis	BRUC020	Live organism / WB	5.00+01 CFU/mL	Not Detected
Brucella suis	BRUC089	Live organism / WB	5.00+02 CFU/mL	Not Detected
Brucella suis	BRUC104	Live organism / WB	5.00+02 CFU/mL	Not Detected
Enterobacter aerogenes	ENTB001	Nucleic Acid / SB	Unknown	Not Detected
Enterobacter agglomerans	ENTB002	Nucleic Acid / SB	Unknown	Not Detected
Francisella novicida	FRAN003	Nucleic Acid / SB	Unknown	Not Detected
Francisella philomiragia	FRAN002	Nucleic Acid / SB	Unknown	Not Detected
Francisella philomiragia	FRAN017	Nucleic Acid / SB	Unknown	Not Detected
Francisella tularensis	FRAN001	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN004	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN012	Live organism / WB	5.00+03 CFU/mL	Not Detected

Organism/Virus	ID #	Sample & Matrix (SB[1] or WB[2])	Concentration	BioThreat-E Result
Francisella tularensis	FRAN016	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN024	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN025	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN031	Live organism / WB	5.00+03 CFU/mL	Not Detected
Francisella tularensis	FRAN072	Live organism / WB	5.00+03 CFU/mL	Not Detected
Haemophilius influenzae	HAEM001	Nucleic Acid / SB	Unknown	Not Detected
Klebsiella pneumoniae	KLEB001	Nucleic Acid / SB	Unknown	Not Detected
Listeria monocytogenes	LIST001	Nucleic Acid / SB	Unknown	Not Detected
Marburg virus	Marbg001	Inactivated organism/ WB	1.00E+05 PFU/mL	Not Detected
Marburg virus	Marbg002	Inactivated organism/ WB	1.00E+05 PFU/mL	Not Detected
Marburg virus	Marbg003	Inactivated organism/ WB	1.00E+05 PFU/mL	Not Detected
Marburg virus	Marbg005	Inactivated organism/ WB	1.00E+05 PFU/mL	Not Detected
Moraxella cattaharalis	MORA001	Nucleic Acid / SB	Unknown	Not Detected
Pasteurella multocida	PAST001	Nucleic Acid / SB	Unknown	Not Detected
Proteus vulgaris	PROT002	Nucleic Acid / SB	Unknown	Not Detected
Providencia stuartii	PROV001	Nucleic Acid / SB	Unknown	Not Detected
Pseudomonas aeruginosa	PSEU001	Nucleic Acid / SB	Unknown	Not Detected
Ralstonia pickettii	RALS001	Nucleic Acid / SB	Unknown	Not Detected
Serratia marcescens	SERR003	Nucleic Acid / SB	Unknown	Not Detected
Shigella flexneri	SHEG001	Nucleic Acid / SB	Unknown	Not Detected
Shigella sonnei	SHEG002	Nucleic Acid / SB	Unknown	Not Detected
Staphylococcus aureus	STAP014	Nucleic Acid / SB	Unknown	Not Detected
Staphylococcus hominis	STAP004	Nucleic Acid / SB	Unknown	Not Detected
Streptococcus pneumoniae	STRE017	Nucleic Acid / SB	Unknown	Not Detected
Streptococcus pyogenes	STRE010	Nucleic Acid / SB	Unknown	Not Detected
VEE virus	Alpha012	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha013	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha014	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha015	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha016	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha017	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha018	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha019	Live organism / WB	1.00E+03 PFU/mL	Not Detected
VEE virus	Alpha020	Live organism / WB	1.00E+04 PFU/mL	Not Detected
VEE virus	Alpha021	Live organism / WB	1.00E+04 PFU/mL	Not Detected
VEE virus	Alpha022	Live organism / WB	1.00E+04 PFU/mL	Not Detected
Yersinia aldovae	YERS098	Nucleic Acid / SB	Unknown	Not Detected
Yersinia enterocolitica	YERS014	Nucleic Acid / SB	Unknown	Not Detected
Yersinia enterocolitica	YERS094	Nucleic Acid / SB	Unknown	Not Detected
Yersinia enterocolitica	YERS095	Nucleic Acid / SB	Unknown	Not Detected
Yersinia frederiksenii	YERS097	Nucleic Acid / SB	Unknown	Not Detected
Yersinia frederiksenii	YERS005	Nucleic Acid / SB	Unknown	Not Detected
Yersinia kirstensenii	YERS096	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pestis	Pending	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS016	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS017	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS018	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS019	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS020	Live organism / WB	2.00E+02 CFU/mL	Not Detected
Yersinia pestis	YERS021	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS022	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS023	Live organism / WB	5.00E+01 CFU/mL	Not Detected

Organism/Virus	ID #	Sample & Matrix (SB[1] or WB[2])	Concentration	BioThreat-E Result
Yersinia pestis	YERS073	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS074	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS078	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS079	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS080	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS082	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pestis	YERS083	Live organism / WB	5.00E+01 CFU/mL	Not Detected
Yersinia pseudotuberculosis	YERS008	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS085	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS086	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS087	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS090	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS091	Nucleic Acid / SB	Unknown	Not Detected
Yersinia pseudotuberculosis	YERS092	Nucleic Acid / SB	Unknown	Not Detected

[1] SB = Sample Buffer

[2] WB = Whole Blood

Note: PFU, plaque forming unit; CFU, colony forming unit

Table 4 Cross-Reactivity Organism Set 2 FilmArray BioThreat-E (v2.4)

Organism/Virus	Strain Information	Concentration	BioThreat-E Result
Acinetobacter baumannii	ATCC 9955	1.00E+08 CFU/mL	Not Detected
Babesia microti	Not Available	Unknown	Not Detected
Bacillus anthracis Vegetative	Not Available	1.00E+07 CFU/mL	Not Detected
Bacillus cereus	E33L	2.00E+07 CFU/mL	Not Detected
Bacillus cereus	03BB 102	1.20E+08 CFU/mL	Not Detected
Borrelia Burgdorferi	Not Available	1.55E+09 GE/mL	Not Detected
Brucella abortus	Not Available	Unknown	Not Detected
Brucella melitensis	Not Available	Unknown	Not Detected
Brucella suis	Not Available	Unknown	Not Detected
Burkholderia mallei/ psuedomallei	Not Available	1.82E+09 GE/mL	Not Detected
Candida albicans	ATCC 10231	1.00E+06 CFU/mL	Not Detected
Candida parapsilosis	ATCC 90875	1.00E+06 CFU/mL	Not Detected
Coxiella burnettii	Not Available	3.80E+09 CFU/mL	Not Detected
Dengue 1	Hawaii	1.60E+10 $TCID_{50}$/mL	Not Detected
Dengue 1	BC89/04 (Costa Rica)	8.90E+03 $TCID_{50}$/mL	Not Detected
Dengue 1	228690 (Jamaica)	1.60E+04 $TCID_{50}$/mL	Not Detected
Dengue 1	276RKI (India)	8.90E+04 $TCID_{50}$/mL	Not Detected
Dengue 1	12150 (Philippines)	1.60E+04 $TCID_{50}$/mL	Not Detected

Organism/Virus	Strain Information	Concentration	BioThreat-E Result
Dengue 1	TH-Sman (no geographical info)	2.80E+05 $TCID_{50}$/mL	Not Detected
Dengue 2	IQT2913 (Peru)	2.80E+05 $TCID_{50}$/mL	Not Detected
Dengue 2	BC100 (Bolivia)	2.80E+07 $TCID_{50}$/mL	Not Detected
Dengue 2	1349 (Burkina Faso, W. Africa)	1.60E+08 $TCID_{50}$/mL	Not Detected
Dengue 2	DakArA1247 (Cote d'Ivoire)	8.00E+04 $TCID_{50}$/mL	Not Detected
Dengue 2	Dakar, Senegal	1.60E+11 $TCID_{50}$/mL	Not Detected
Dengue 2	New Guinea C (New Guinea)	6.00E+01 $TCID_{50}$/mL	Not Detected
Dengue 2	10674 (Dakar, Senegal)	8.00E+03 $TCID_{50}$/mL	Not Detected
Dengue 2	S-14635 (Tonga)	8.90E+04 $TCID_{50}$/mL	Not Detected
Dengue 2	BC102/94 (Saudi Arabia)	2.80E+04 $TCID_{50}$/mL	Not Detected
Dengue 2	K0049 (Kamphaeng Phet, Thailand)	2.80E+07 $TCID_{50}$/mL	Not Detected
Dengue 2	429557 (Mexico)	8.90E+06 $TCID_{50}$/mL	Not Detected
Dengue 2	P8-1407MS (monkey in Malaysia)	2.30E+06 $TCID_{50}$/mL	Not Detected
Dengue 2	S-40921 (Burma)	8.90E+05 $TCID_{50}$/mL	Not Detected
Dengue 2	BC27/96 (Vietnam)	1.60E+08 $TCID_{50}$/mL	Not Detected
Dengue 2	BC171-96 (Philippines)	2.80E+07 $TCID_{50}$/mL	Not Detected
Dengue 2	BC141-96 (Puerto Rico)	8.90E+05 $TCID_{50}$/mL	Not Detected
Dengue 2	PM33974 (Guinea)	8.00E+04 $TCID_{50}$/mL	Not Detected
Dengue 2	ArArA1247 (Burkina Faso, W. Africa)	8.00E+04 $TCID_{50}$/mL	Not Detected
Dengue 2	DEN2-S2 (New Guinea)	1.60E+06 $TCID_{50}$/mL	Not Detected
Dengue 3	BC14/97 (Malaysia)	8.90E+05 $TCID_{50}$/mL	Not Detected
Dengue 3	271242 (Sri Lanka)	1.60E+04 $TCID_{50}$/mL	Not Detected
Dengue 3	Phillipines/H87/1956	4.45E+02 $TCID_{50}$/mL	Not Detected
Dengue 3	MK-594-87 (Thailand)	1.60E+04 $TCID_{50}$/mL	Not Detected
Dengue 3	S-40580 (Burma)	1.60E+06 $TCID_{50}$/mL	Not Detected
Dengue 3	BC188/97 (Mexico)	2.80E+05 $TCID_{50}$/mL	Not Detected
Dengue 4	H241 (no geographical info)	1.60E+08 $TCID_{50}$/mL	Not Detected
Dengue 4	BC13/97 (Malaysia)	1.60E+06 $TCID_{50}$/mL	Not Detected
Dengue 4	BC258/97 (Puerto Rico)	2.80E+05 $TCID_{50}$/mL	Not Detected
Dengue 4	D85-019 (Thailand)	1.60E+05 $TCID_{50}$/mL	Not Detected
Dengue 4	BC287/97 (Mexico)	2.80E+06 $TCID_{50}$/mL	Not Detected
Dengue 4	BC123/97 (Malaysia)	2.80E+05 $TCID_{50}$/mL	Not Detected
Enterobacter cloacae	ATCC 13047	1.00E+08 CFU/mL	Not Detected
Enterococcus faecalis, vanB+	JMI 368	1.00E+08 CFU/mL	Not Detected
Escherichia coli	ATCC 43888	1.00E+08 CFU/mL	Not Detected
Francisella tularensis	Not Available	1.00E+06 Cells/mL	Not Detected
Haemophilus influenzae	ATCC 10211	1.00E+08 CFU/mL	Not Detected

Organism/Virus	Strain Information	Concentration	BioThreat-E Result
Herpes simplex virus-1	Not Available	3.05E+07 GE/mL	Not Detected
Herpes simplex virus-2	Not Available	3.00E+07 GE/mL	Not Detected
Influenza A Virus	H3N2	1.70E+04 $TCID_{50}$/mL	Not Detected
Klebsiella oxytoca	ATCC 13182	1.00E+08 CFU/mL	Not Detected
Klebsiella pneumoniae	ATCC 13883	1.00E+08 CFU/mL	Not Detected
Leishmania chagasi	Not Available	2.10E+05 Cells/mL	Not Detected
Leishmania donovani	Not Available	5.05E+05 Cells/mL	Not Detected
Leishmania infantum	Not Available	1.98E+05 Cells/mL	Not Detected
Leptospira biflexa	Not Available	Unknown	Not Detected
Leptospira interrogans	Not Available	Unknown	Not Detected
Marburgvirus	RAVN	2.50E+06 PFU/mL	Not Detected
Orientia tsutsugamushi	112603	1.00E+03 GE/mL	Not Detected
Plasmodium falciparum	3D7	1.67E+06 Cells/mL	Not Detected
Plasmodium falciparum	D6	1.70E+02 Cells/mL	Not Detected
Plasmodium falciparum	Uganda Palo Alto	1.70E+02 Cells/mL	Not Detected
Plasmodium falciparum	Malayan Camp R+	1.70E+02 Cells/mL	Not Detected
Plasmodium malariae	gBlock, IDT	3.33E+03 GE/mL	Not Detected
Plasmodium ovale	gBlock, IDT	3.33E+07 GE/mL	Not Detected
Plasmodium vivax	gBlock, IDT	3.33E+07 GE/mL	Not Detected
Plasmodium falciparum	Ghana III	1.70E+04 Cells/mL	Not Detected
Plasmodium falciparum	RO33	1.70E+02 Cells/mL	Not Detected
Plasmodium falciparum	106-1	1.70E+02 Cells/mL	Not Detected
Proteus mirabilis(CFU)	ATCC 29906	1.00E+08 CFU/mL	Not Detected
Pseudomonas aeruginosa	ATCC 27853	1.00E+08 CFU/mL	Not Detected
Rickettsia conorii	Not Available	1.00E+03 CFU/mL	Not Detected
Rickettsia prowazeckii	Not Available	5.66E+06 CFU/mL	Not Detected
Rickettsia rickettsi	Not Available	1.00E+03 GE/mL	Not Detected
Rickettsia typhi	Not Available	1.00E+03 GE/mL	Not Detected
Salmonella enterica serovar Typhimurium	ATCC 14028	5.40E+10 CFU/mL	Not Detected
Serratia marcescens	ATCC 27137	1.00E+08 CFU/mL	Not Detected
Shigella flexneri	Not Available	1.00E+09 GE/mL	Not Detected
Staphylococcus aureus	ATCC 11632	1.00E+08 CFU/mL	Not Detected
Staphylococcus epidermidis	ATCC 12228	1.00E+08 CFU/mL	Not Detected
Streptococcus pneumoniae	ATCC BAA-255	1.00E+08 CFU/mL	Not Detected
Trypanosoma cruzi (Cells)	Not Available	3.75E+05 Cells/mL	Not Detected
Yellow Fever Virus (TCID50)	Not Available	4.45E+06 $TCID_{50}$/mL	Not Detected
Yersinia enterocolitica	Not Available	1.00E+02 GE/mL	Not Detected
Yersinia pestis	Not Available	9.78E+07 CFU/mL	Not Detected
Yersinia pseudotuberculosis	Not Available	2.00E+03 GE/mL	Not Detected

Based on the results of these two analytical specificity studies, the FilmArray BioThreat-E v2.5) test is not expected to cross-react with near neighbors, other biothreat organisms, or other organisms found in blood from individuals with fever.

Contrived Clinical Specimen Study Using Inactivated Ebola Zaire Virus

The estimated LoD of the FilmArray BioThreat-E test for inactivated Ebola Zaire Mayinga virus was 6.00 E+05 PFU/ml. To predict clinical performance, 25 independent whole blood, and 25 independent urine specimens were spiked with inactivated Ebola Zaire Mayinga virus at the concentrations listed in Table 5 below. In addition 25 un-spiked whole blood and 25 un-spiked urine specimens were also tested.

Table 4. Contrived Specimen Spiking Scheme

Spike Level-Blood	Number	Spike Level-Urine	Number
No virus	25	No virus	25
2 × LoD (1.2 E+06 PFU/mL)	20	2 × LoD (1.2 E+06 PFU/mL)	20
2.5 x LoD (1.5 E+06 PFU/mL)	5	2.5 x LoD (1.5 E+06 PFU/mL)	5
Total	50	Total	50

Contrived specimens were randomized and analyzed with the FilmArray BioThreat-E test. The blinded spiking key was unmasked after valid runs were obtained for all 100 specimens. The results of this study are summarized in Table 6 (whole blood) and Table 7 (urine) below.

Table 6. Contrived Whole Blood Specimens Study Summary Results

BioThreat-E Detection	**Detected**	**Not Detected**	
Positive Specimens (2x LoD) (20)	19	1	
Positive Specimens (2.5xLoD) (5)	5	0	
Negative Specimens (25)	0	25	
			95% CI
Positive Percent Agreement	24/25	96.0%	80.5-99.3%
Negative Percent Agreement	25/25	100%	86.7-100%

Table 7. Contrived Urine Specimens Study Summary Results

BioThreat-E Assay Detection	Detected	Not Detected	
Positive Specimens (2x LoD) (20)	19	1	
Positive Specimens (2.5x LoD) (5)	5	0	
Negative Specimens (25)	0	25	
			95% CI
Positive Percent Agreement	24/25	96.0%	80.5-99.3%
Negative Percent Agreement	25/25	100%	86.7-100%

FilmArray BioThreat-E test correctly identified 24/25 whole blood and 24/25 urine specimens spiked with inactivated Ebola Zaire at the concentrations shown above, including concentrations near the limit of detection. No false positive results were detected.

Points of Contact

Contact information for technical assistance for the FilmArray BioThreat-E Test:

BioFire Defense Technical Assistance

79 W 4500 S, Suite 14
Salt Lake City, UT 84107
Phone: 1-801-262-3592
Fax: 1-801-447-6907
support@biofiredefense.com

Health care providers will be contacted by BioFire Defense, LLC in the event of any significant new findings observed during the course of the emergency use of the BioThreat-E Test.

Any adverse events should be reported to the following website:

http://biofiredefense.com/support/filmarray-support/BioThreat-E Report